REMARKS

Reconsideration and withdrawal of the rejections of the application are respectfully requested in view of the remarks, amendments, and enclosures herewith. Applicants thank Examiner Nashed, his SPE, and Practice Specialist Eyler for the any courtesies extended in the October 6, 2005 telephonic interview.

I. STATUS OF THE CLAIMS

Claims 1-3, 9-15 and 30-38 are now pending. Claims 1 and 33 have been amended, claims 4, 6 and 7 have been cancelled, and new claims 37 and 38 have been added, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents.

No new matter is added.

It is submitted that the claims, as originally presented and as herein presented, are patentably distinct over the prior art cited by the Examiner, and that these claims are in full compliance with the requirements of 35 USC 112. The new claim, as presented herein, is not submitted for the purpose of patentability within the meaning of 35 USC sections 101, 102, 103 or 112. Rather, this new claim is submitted simply for clarification and to round out the scope of protection to which Applicants are entitled.

II. THE REJECTIONS UNDER 35 U.S.C. §112 ARE OVERCOME

Claims 1-4, 6-7, 9-15, 30-32 and 34-36 were rejected under 35 U.S.C. §112, first paragraph, as allegedly subject matter which was not described in the specification in such a way as to reasonably convey that Applicants were in possession of the invention at the time of filing.

Claims 1-4, 6-7, 9-15, 30-32 and 34-36 were rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement for any hexose oxidase and any substrate other than *Chondrus cripus* hexose oxidase of SEQ ID NO: 2 and those substrates specifically identified in the specification. The rejection is respectfully traversed.

It is respectfully submitted that the amendments to claim 1 herein have rendered these rejections moot.

Claim 33 was rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite. The rejection is respectfully traversed.

It is respectfully submitted that claim 33 has been amended herein to accurately recite SEQ ID NO:2 instead of SEQ ID NO: 1. Accordingly, the rejection is now moot.

Reconsideration and withdrawal of the rejections under 35 U.S.C. §112 is respectfully requested.

III. THE REJECTIONS UNDER 35 U.S.C. §103 ARE OVERCOME

Claims 1-4, 6-7, 9-15 and 30-37 were rejected under 35 U.S.C. §103 as allegedly being unpatentable over Hamade *et al.* in view of Hansen *et al.*, and in view of the "known fact in the art that glucose can be obtained by the action of amyloglucosidase on starch".

Additionally, claims 1-4, 6-7, 9-15, 30-32 and 34-36 were rejected under 35 U.S.C. §103 as allegedly being unpatentable over Hamade *et al.* in view of Stougaard *et al.* and in view of the "known fact in the art that glucose can be obtained by the action of amyloglucosidase on starch".

Applicants respectfully traverse the rejections and will address them collectively.

Applicants respectfully assert that the Hamade reference used in repeatedly rejecting the case has been repeatedly misunderstood, and that Hamade does not anticipate or render obvious the present invention, either alone or in any combination.

For example, in speaking to the alleged teachings of Hamade, the Office Action states, "In addition, they teach that the substrate of said oxidase can be produced within the composition by a second enzyme action on a precursor substrate such as the action of cellulase, chitonase, and lysothyme on chitosan to produce glucose, see page 5, lines 50-54." Office Action at page 8.

As discussed in the October 6, 2005 telephonic interview, for which Examiner Nashed, his SPE, and Practice Specialist Eyler are thanked for the courtesies extended therein, attached is an extract from Food Polysaccharides which shows the structure of chitosan and clearly states (see page 442) that chitosan contains glucosamine units and N-acetylglucosamine units, i.e. NO glucose units are present. Also attached are portions of the Sigma Aldrich website, showing that all enzymes mentioned by the Examiner act by hydrolyzing various 1,4-Beta linkages, such that, if reacted with chitosan, would NOT provide glucose units.

As we believe we have shown during prosecution heretofore, and as we will gladly further show by reference to the attached documents, inter alia, the quoted statement above is just plain incorrect; chitosan does NOT degrade to give glucose. Therefore, it is respectfully submitted that the application has been erroneously repeatedly rejected on the basis of Hamade.

During the October 6, 2005 telephonic interview, it was suggested that the disclosure at page 3, lines 40-41, in Hamade *et al.* suggests compositions such as those claimed in the present invention. Applicants respectfully disagree. It is respectfully submitted that the skilled artisan would actually understand the disclosure in Hamade *et al.* at page 3, lines 40-41, to be referring to the situation where a naturally occurring enzymatic or chemical reaction spontaneously runs as a second reaction. Spontaneous second reactions such as this are well known in systems using enzymes. For example, hexose oxidase can react with certain substrates to form gluconodeltalactone. In water, this gluconodeltalactone spontaneously undergoes hydrolysis to gluconic acid. An alternative example is observed following the addition of ascorbic acid to dough: this ascorbic acid is transformed to dehydroascorbic acid by ascorbate oxidase present in flour. Furthermore, it is noted that despite discussing a very wide range of possible substrates and enzymes, Hamade *et al.* does not provide a single disclosure of a system using more than one enzyme. Thus, it is respectfully submitted that while Hamade *et al.* does provide a suggestion that a substrate may go through more than one step to produce an antimicrobial compound, Hamade does not disclose or suggest any compositions comprising more than one enzyme.

Furthermore, the present invention requires the presence of a first substrate (which is a carbohydrate or a sugar) that reacts with a first enzyme to form a second substrate selected from the group consisting of D-glucose, D-galactose, maltose, lactose and cellobiose. Thus, the first substrate must be a polymeric sugar or carbohydrate comprising at least one of the required structural units of the second substrate. Therefore, the invention requires that the first enzyme reacts with a polymeric first substrate to produce a second substrate on which the second enzyme is active. In contrast, the only disclosure of an enzyme being active on a polymeric species in Hamade *et al.* relates to the action of a chitosan-decomposing enzyme on citosan to produce a compound having antimicrobial activity as a direct decomposition product of chitosan (see EP0866103, claim 13). Such a system actually teaches away from carrying out a second reaction with a second enzyme on the product formed from the reaction of the polymeric species with a first enzyme.

It is also noted that Hamade *et al.* provides a wide list of non-limiting possible enzyme-substrate combinations which can generate a large number of different antimicrobial agents. No directions are given that would lead a skilled person to select a particular combination over any

other combination that is mentioned in the specification. For example, when referring to the production of hydrogen peroxide as the antimicrobial agent, we note that Hamade provides a wash list of enzyme-substrate combinations, see page 5, lines 14-44, (EP0866103). Furthermore, Hamade *et al.* also provides details of the particular types of reactions which can produce hydrogen peroxide, see page 5, lines 26-44. This appears to cover all of the possibilities envisaged by the inventors, as there is no disclosure or suggestion of using a first substrate which reacts with a first enzyme to form a second substrate which reacts with hexose oxidase as required by the present invention. Instead, this passage of Hamade *et al.* continually reiterates that a single enzyme reacts with a single substrate to form the desired antimicrobial agent, hydrogen peroxide.

In relation to the antimicrobial compound, Hamade *et al.* also states,

> Therefore, even when this compound is highly soluble, unstable, or hard to handle, the inherent antimicrobial activity of the compound can be fully exploited. For example, it is by now possible to previously convert a highly water-soluble compound to an insoluble compound, ..., then incorporate the resulting compound in a matrix beforehand, and cause the objective compound to be produced by an enzymatic reaction.
> EP0866103, page 6, line 19-24.

Thus, a key feature for the practical application of the invention of Hamade *et al.* is that the substrate is insoluble whereas the antimicrobial compound is soluble. This feature prevents the substrate from leaching out of the paint into the water. The importance of this feature can be readily seen from the actual examples of Hamade *et al.* Thus, the substrates tributyrin (Example 1), cholesterol (Example 2 and 3) and tricaprin (Example 4) are all insoluble in water (see attached product information sheets). However, it would be readily apparent to the skilled person that many of the suggested enzyme-substrate combinations do not contain this feature. For example, glucose is water soluble, thus the combination of hexose oxidase-glucose mentioned in Hamade *et al.* would not actually provide a solution to the problem of controlled release. Thus, we submit that such combinations would be discounted by the skilled person as not being capable of solving the problem of controlled release of the antimicrobial compound.

In addition, while Hamade *et al.* mention the problem of achieving controlled release of the compound having antimicrobial activity, they suggest that this problem is solved merely by

dispersing the enzyme and the substrate in a matrix (see EP 0866103, page 6, lines 3-12). In particular, Hamade *et al.* states:

> In the present invention, the penetration of water into the matrix occurs gradually and sustainedly so that the compound having antimicrobial activity is produced persistently at a controlled rate, thus achieving controlled release of this compound. (EP0866103, page 6, line 10-12).

Hamade *et al.* go on to state that the problem of controlled release can be easily reduced to practice through the use of a coating composition of the invention. This coating composition "comprises a film-forming resin, an enzyme, and a substate, said enzyme being capable of reacting with said substate to produce a compound having antimicrobial activity." (EP0866103, page 6, line 25-30).

Thus, these statements discussed above, as made in Hamade *et al.*, teach away from the present invention as they suggest that a composition comprising an enzyme, a substrate and a film-forming resin is sufficient to overcome the problem of controlled release of the antimicrobial agent. There is no suggestion in these disclosures of using a composition according to the present invention. Nor, given the disclosure in Hamade *et al.* would the skilled artisan have any motivation or expectation of success to adapt the teachings of this document to produce the present invention.

Accordingly, reconsideration and withdrawal of the rejections under 35 U.S.C. §103 is respectfully requested.

REQUEST FOR INTERVIEW

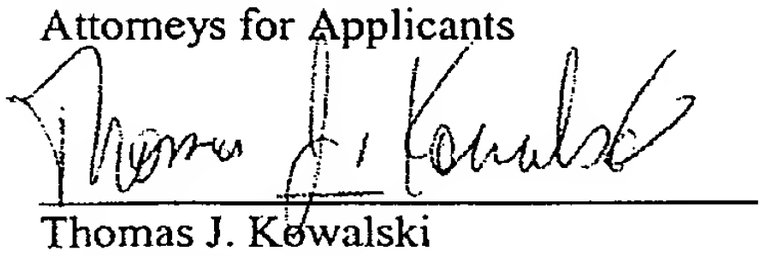
If any issue remains as an impediment to allowance, a further interview with the Examiner and his supervisor is respectfully requested, prior to issuance of any paper other than a Notice of Allowance; and, the Examiner is respectfully requested to contact the undersigned to arrange a mutually convenient time and manner for such an interview.

PATENT
674509-2035

CONCLUSION

In view of the amendment and remarks herewith, the application is in condition for allowance. Favorable reconsideration of the application and prompt issuance of a Notice of Allowance, or an interview at a very early date with a view to placing the application in condition for allowance, are earnestly solicited. The undersigned looks forward to hearing favorably from the Examiner at an early date.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP
Attorneys for Applicants

By: 

Thomas J. Kowalski
Reg. No. 32,147
Angela M. Collison
Reg. No. 51,107
Tel (212) 588-0800
Fax (2120 588-0500

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FOOD POLYSACCHARIDES and Their Applications

edited by

Alistair M. Stephen
Department of Chemistry
University of Cape Town
Rondebosch, South Africa

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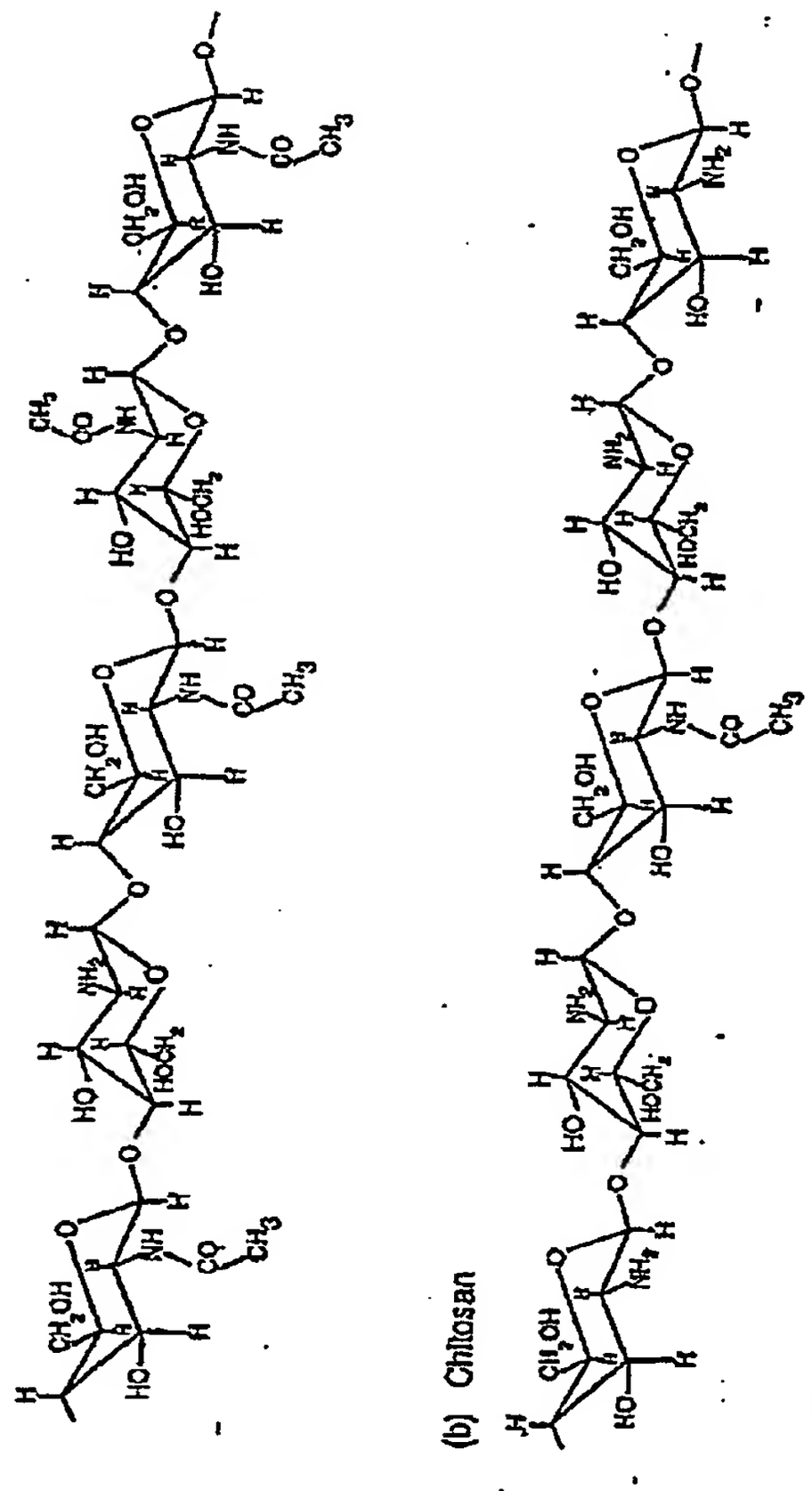


Figure 1 Molecular structures of (a) chitin and (b) chitosan.

papers describe bioapplications of chitin and chitosan, such as medical, cosmetic, agricultural, and food-related uses. Although not always accessible, the enormous abundance of chitin and its unusual chemical properties, which can readily be modified, are factors underlying current interest in developing its commercial potential.

B. Molecular Structures

The elucidation of the molecular structures of chitin and chitosan has literally taken centuries to accomplish. In fact, some of the finer details are still being discovered. Braconnot understood as early as 1811 that chitin contained nitrogen [1], and in 1878 Ledderhose wrote that acetic acid and glucosamine were hydrolysis products of chitin [26]. It was not until the 1950s that there was consensus within the scientific community that chitin was a polysaccharide consisting of small proportions of glucosamine units and large amounts of *N*-acetylated glucosamine [27]. Today it is recognized that most commercially prepared chitin is a linear copolymer composed of approximately 70–90% *N*-acetyl-D-glucosamine and 10–30% D-glucosamine units [28], connected through (1→4)-linked β-glycosidic linkages [29]. Most commercial grades of chitosan contain 75–95% glucosamine and 5–25% *N*-acetylglucosamine units [28]. The molecular weight of these polysaccharides can be as high as 10^6 [30], unless some special treatment is used to degrade them. Results of experiments based on lysozyme degradation reactions [31] and nuclear magnetic resonance (NMR) spectroscopy [32] have suggested that the two different units are distributed randomly and are not blocked together (Fig. 1).

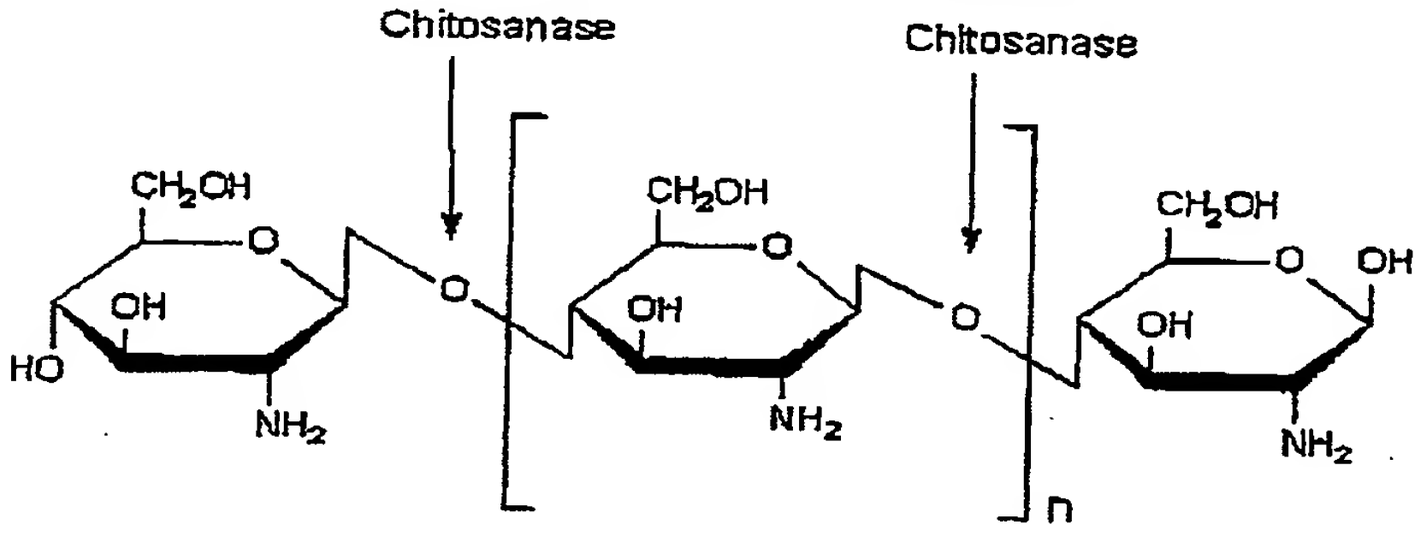
Chitin and chitosan are also known to exhibit polymorphism [33]. Generally, the individual chains assume an essentially linear structure, which undergoes one full twist every 10.1–10.5 Å along the chain axis. Since each glycosidic unit in the chain is chiral and all units are connected by an oxygen atom that links C1 of one glycosidic unit to C4 of an adjacent unit, a distinct "left" and "right" direction can be assigned to each polymer chain. The most common allomorph exhibited by chitin and chitosan is known as the α conformation, in which the unit cell is orthorhombic [29] and the individual polymer chains are believed to be arranged in an antiparallel fashion. Thus, adjacent chains are oriented in opposite directions. A less common allomorph, known as the β conformation, is thought to correspond to a monoclinic unit cell [34] with the polymer chains arranged in a parallel fashion. X-ray diffraction patterns and NMR spectra of these two different allomorphs have been shown to be distinct [35]. Other allomorphs have also been identified. Figure 2 shows an illustration of the parallel and antiparallel polymer chain arrangements.

C. Chemical and Physical Properties

Although the molecular structures of chitin and chitosan seem quite similar, the chemical reactions they undergo and their physical properties are often surprisingly distinct. Both compounds possess reactive hydroxyl and primary amine groups, but chitosan is usually less crystalline than chitin, which presumably makes chitosan more accessible to reagents. Chitosan also has the higher concentration of primary amine groups, which makes it more nucleophilic and basic. Upon heating, both compounds decompose prior to melting. Thus, these polymers have no melt point. Since a complete description of the chemical and physical properties of these polysaccharides is beyond the scope of this text, the information presented herein will be limited to a few common organic reactions, properties of heavy metal complexes, enzymatic reactions, and solution properties of these polymers. For a more detailed description the reader is referred to works by Muzzarelli [33], Zikakis [36], Tolnra

Chitosanase and Chitosan

Chitosanase Specificity



Chitosan

Polymer of β-(1-4)-D-glucosamine units

Chitosanase
EC# 3.2.1.132
Synonyms: chitosan N-acetylglucosaminohydrolase

Chitosanase catalyzes the endohydrolysis of β-1,4-linkages between D-glucosamine (GlcN-GlcN) residues in chitosan. The enzyme from Streptomyces has been reported to also hydrolyze the GlcNAc-GlcN linkage in partially acetylated chitosan.

from *Streptomyces species*
Product Number C0794 (glycerol solution, min 15 un/mg)

from *Streptomyces griseus*
Product Number C9830 (lyophilized, >50 un/mg)

References
2. *Enzyme Nomenclature* (www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/132.html)
3. Fukamizo, T., et al., Reaction mechanism of chitosanase from Streptomyces sp. N174,. Biochem. J., 311, 377-83 (1995)

Cellulase
EC# 3.2.1.4
Synonyms: 1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase

from *Aspergillus species*
Product Number C2605 (Novozymes Carezyme 1000 L)

from *Aspergillus niger*
Product Number C1184 (powder, min. 0.3 un/mg)

from *Trichoderma reesei*
Product Number C8546 (powder, min. 1.0 un/mg)
Product Number C2730 (Novozymes Celluclast 1.5 L)

from *Trichoderma viride*
Product Number C0615 (Yakult Onozuka RS)
Product Number C1794 (cell culture tested, powder, min. 3.0-10 un/mg)
Product Number C9422 (powder, min. 3.0-10 un/mg)

Cellulase catalyzes the endohydrolysis of 1,4-b-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans[1]

Driselase
from *Basidiomycetes sp.*
Product Number D9515 (Crude powder containing laminarinase, xylanase and cellulase)

Lysozyme
EC# 3.2.1.17
Synonyms: peptidoglycan N-acetylmuramoylhydrolase

Lysozyme catalyzes the hydrolysis of 1,4-β-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins

from chicken egg white
Product Number L6876 (lyophilized, approx 50,000 un/mg)
Product Number L7651 (molecular biology grade, lyophilized, approx 50,000 un/mg)
Product Number L7001 (lyophilized, approx 50,000 un/mg)
Product Number L6876 (Asceptically filled, lyophilized, approx 50,000 un/mg)
Product Number L2879 (chloride, lyophilized, approx 60,000 un/mg)
Product Number L2879 (immobilized on agarose)
Product Number L0289 (biotin-caproyl, lyophilized, > 20,000 un/mg)

from human milk
Product Number L7001 (lyophilized, minimum 100,000 un/mg)
Product Number L1667 (recombinant, expressed in rice, lyophilized, minimum 100,000 un/mg)

from human neutrophils
Product Number L8402 (lyophilized, minimum 100,000 un/mg)

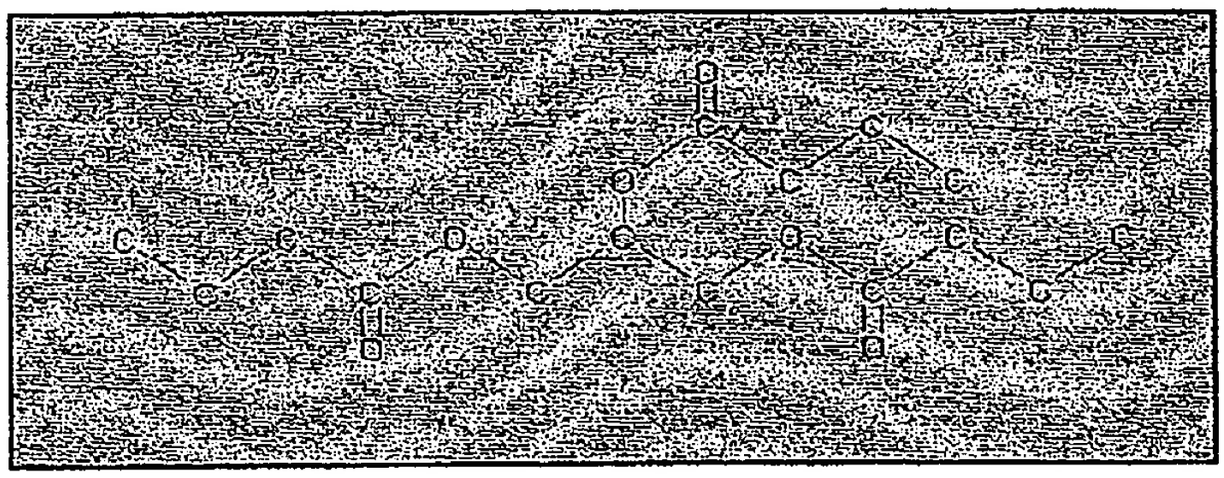
References
1. *Enzyme Nomenclature* (www.chem.qmul.ac.uk/iubmb/enzyme/EC3/2/1/17.html)

Tributyrin

NSC 661583

Structure

If clicking on the 2D structure does not bring up the 3D structure, you need to configure your browser to handle Chemical MIME types



Names

- Tributyrin
- 1,2,3-Tributyrylglycerol
- Glyceryl tributyrate

Chemical Data

CAS Registry Number: 60-01-5

Molecular Formula: $C_{15}H_{26}O_6$

Molecular Weight: 302

Approximate Solubility

Virtuall insoluble in water. Freely soluble in common organic solvents such as ethanol and methanol

Stability

Liquid bulk tributyrin is stored at room temperature. Formal stability studies have yet to be conducted

Ultraviolet Absorption

End absorption only

High Performance Liquid Chromatography

- Column: Zorbax C8 250 mm × 4.6 mm i.d.
- Mobile Phase: Acetonitrile:water 70:30
- Flow Rate: 1.5 mL/min
- Detection: UV at 210 nm
- Sample Preparation: Internal standard solution is added to 50 mg of the sample in a 5 mL volumetric flask to bring to volume
- Internal Standard: Octanophenone, 0.2 mg/mL in acetonitrile
- Retention Volume: 11.6 mL (Tributyrin), 19.5 mL(I.S.)

Pharmaceutical Data

3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Cholesterol

Product Number **C 8667**
Storage Temperature -20 °C

Product Description
Molecular Formula: $C_{27}H_{46}O$
Molecular Weight: 386.7
CAS Number: 57-88-5
Melting Point: 147 to 150 °C.[1]
Specific Rotation: $[\alpha]^{25}_{D}$= -34 to -38°
(25 g/100 ml dioxane).[1]

Sheep's wool is used to prepare the wool grease that is the source for this product. It is purified from 95% cholesterol (Product No. C 8503).

A description of isolation and assay methods for cholesterol has been described.[2]

Cholesterol is a component used for the preparation and study of artificial model membranes. One example is a study of valinomycin induced changes in membrane potentials of red blood cell and phospholipid (phosphatidylcholine from egg yolk plus cholesterol) vesicle suspensions. Positively charged cyanine dyes were used that fluorimetrically respond to the change in potential.[3]

Cholesterol is precipitated by digitonin and gives an intense red color with rosaniline in chloroform solution.[4]

Precautions and Disclaimer
For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions
Cholesterol monohydrate is practically insoluble in water (less then 0.5 mg/100 ml of water); slightly soluble in alcohol (1.29% w/w at 20 °C), more soluble in hot alcohol (100 g of saturated 96% alcoholic solution contains 28 g at 80 °C). Also one gram dissolves in 2.8 ml of ether, in 4.5 ml of chloroform, and in 1.5 ml of pyridine. The product is soluble in acetone, dioxane, ethyl acetate, benzene, petroleum ether, oils, fats, and in aqueous solutions of bile salts. Solutions should be protected from light.[4,5,6]

References

1. The United States Pharmacopeia XXI, p. 1550
2. Stadtman, T.C., Preparation and Assay of Cholesterol and Ergosterol. Methods in Enzymology, 3(63), 392-394 (1957).
3. Sims, P.J., et al., Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidylcholine vesicles. Biochemistry 13(16), 3315-3330 (1974).
4. The Merck Index, 11th ed., Entry# 2204
5. Martindale The Extra Pharmacopoeia, 29th ed., Reynolds, J. E. F., ed., The Pharmaceutical Press (London, England: 1989), p. 1324.
6. HANDBOOK OF LIPID RESEARCH, 4, 406 (1988).

RLG/NSB 12/04

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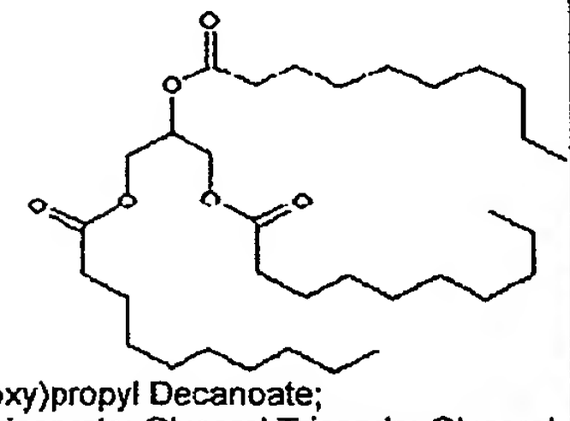

PRODUCT IDENTIFICATION

CAS NO.	621-71-6
EINECS NO.	210-702-0
FORMULA	$C_{33}H_{62}O_6$
MOL WT.	554.84
H.S. CODE	
DERIVATION	
CLASSIFICATION	
TOXICITY	
SYNONYMS	Tri-n-Caprin; 2,3-Bis(decanoyloxy)propyl Decanoate; Tri-Decanoin; Capric acid triglyceride; Caprin; Glycerol Tricaprate; Glycerol Tricaprin; Glycerol Tridecanoate; Tricapric glyceride; Tridecanoin; Glyceryl Tridecanoate; 1,2,3-Tricaprinoylglycerol;

PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL STATE	pale yellow solid
MELTING POINT	33 C
BOILING POINT	
SPECIFIC GRAVITY	0.92
SOLUBILITY IN WATER	Insoluble
pH	
VAPOR DENSITY	
AUTOIGNITION	
NFPA RATINGS	Health: 1 Flammability: 0 Reactivity: 0
REFRACTIVE INDEX	
FLASH POINT	
STABILITY	Stable under ordinary conditions

GENERAL DESCRIPTION AND APPLICATIONS

Glycerol Fatty Acid Esters are used as emulsifiers or oiling agents for foods, spin finishes and textiles; antifoaming and antistatic agents for plastics; and lubricants, water treatment, metal working fluids, and dispersing agents. End applications include cosmetics, foods, personal care products, medicine , pesticides, paper making, plastics and paints. Tricaprin can be used in personal care products and cosmetics for the applications of emollient and cooling effect to the skin. It gives a long lasting non-greasy feel on skin and lips.

SALES SPECIFICATION

APPEARANCE	pale yellow solid
SAP VALUE	295 - 315
ACID VALUE	0.1 max
COLOR APHA	100 max
CHAIN DISTRIBUTION	C6:0 1.0% max + C8:0 5.0% max + C10:0 90.0% min + C12:0 & Higher 5.0% max
MOISTURE	0.1% max
TRANSPORTATION	
PACKING	190kgs in drum
HAZARD CLASS	
UN NO.	
OTHER INFORMATION	

We are pleased to receive your any questions or remarks. You may contact us at the following numbers and e-mail address for additional information.

Fax : + 82 + 2 + 783-8063 , + 82 + 2 + 3775-3073 (Seoul, Korea)